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Effect of Thermal Additions on the Density and Distribution of Thermophilic Amoebae and Pathogenic *Naegleria fowleri* in a Newly Created Cooling Lake

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Pathogenic *Naegleria fowleri* is the causative agent of fatal human amoebic meningoencephalitis. The protozoan is ubiquitous in nature, and its presence is enhanced by thermal additions. In this investigation, water and sediments from a newly created cooling lake were quantitatively analyzed for the presence of thermophilic amoebae, thermophilic *Naegleria* spp., and the pathogen *Naegleria fowleri*. During periods of thermal additions, the concentrations of thermophilic amoebae and thermophilic *Naegleria* spp. increased as much as 5 orders of magnitude, and the concentration of the pathogen *N. fowleri* increased as much as 2 orders of magnitude. Concentrations of amoebae returned to prior thermal perturbation levels within 30 to 60 days after cessation of thermal additions. Increases in the thermophilic amoeba concentrations were noted in Savannah River oxbows downriver from the Savannah River plant discharge streams as compared with oxbows upriver from the discharges. Concentrations of thermophilic amoebae and thermophilic *Naegleria* spp. correlated significantly with temperature and conductivity. Air samples taken proximal to the lake during periods of thermal addition showed no evidence of thermophilic *Naegleria* spp. Isoenzyme patterns of the *N. fowleri* isolated from the cooling lake were identical to patterns of *N. fowleri* isolated from other sites in the United States and Belgium.

Fatal human primary amoebic meningoencephalitis resulting from infection with the amoeba flagellate *Naegleria fowleri* is associated with exposure of the host to heated water (2–5, 9, 11). Increased concentrations of *N. fowleri* are associated with either artificially or naturally heated aquatic habitats (6, 8, 13, 14, 21, 22, 25; B. S. Robinson and J. A. Lake, paper presented at the 9th federal convention of the Australian Water and Wastewater Convention, Perth, Australia, 6 to 10 April, 1981).

Isolation of pathogenic *Naegleria* spp. from thermally altered lakes and rivers in Florida, Georgia, and Virginia and from man-made lakes in Texas, Minnesota, and Illinois that received electrical power plant effluents has been reported. In a study by Stevens et al. (20) *N. fowleri* was not isolated from any of seven naturally heated sites with temperatures ranging from 30 to 34°C. However, the pathogen was isolated from waters receiving thermal discharges from electric power plants at 3 of the 13 thermally altered sites with temperatures ranging from 35 to 41°C.

A detailed study of the distribution of thermophilic and pathogenic *Naegleria* spp. in thermal discharges of northern and southern electric power plants relative to ambient source waters has been documented (21, 22). Of the 385 samples from 17 cooling systems, 195 were positive for thermophilic amoebae (51%). Of the 195 thermophilic amoeba isolates from the power plant cooling systems, 85 (44%) were identified as *Naegleria* spp. by the flagellation test and from morphological criteria. Of the 85 identified, 16.5% were *N. fowleri*, whereas the remaining isolates were probably *Naegleria lovaniensis*.

In contrast to results from the cooling waters, in unheated control waters only 5% of the amoeba isolates were *Naegleria* spp., and none of these were pathogenic. Data from the 17 test sites supported the association between thermophilic, pathogenic amoebae and artificially heated water. Outgrowth of thermophilic amoebae, presence of thermophilic *Naegleria* spp., and isolation of *N. fowleri* in heated versus unheated systems were all significant at the $P < 0.05$ level by chi-square analysis.

An artificially heated lake in Virginia was the first site studied before and during thermal enrichment (7). The presence of *N. fowleri* was related to the initiation of thermal additions to the lake. The pathogen was not isolated before thermal additions; however, *N. fowleri* was isolated after thermal additions began. Increased numbers of amoeboflagellates growing at 44°C were also isolated.

Similar data were reported for two northern impoundments sampled in 1983 (21). One site had received thermal additions from an electric power plant since 1970, and water temperatures at the sampling locations ranged from 34.8 to 41.5°C. The percentages of water and detrital samples that tested positive for the presence of thermophilic amoebae, thermophilic *Naegleria* spp., and *N. fowleri* were 100, 76, and 41, respectively.

The second study site was an unheated lake approximately 185 mi (ca. 298 km) from the heated site. The percentages of samples positive for thermophilic amoebae and thermophilic *Naegleria* spp. were 57 and 27, respectively. *N. fowleri* was not isolated from this site. Statistical comparison between the heated and unheated sites showed significant differences in the presence of thermophilic amoebae, thermophilic *Naegleria* spp., and *N. fowleri* at the $P < 0.01$ level.

In addition to industrial sites, swimming pools, natural hot springs, and solar heated ponds with thermal additions have provided habitats for the survival and growth of *N. fowleri*. Pathogenic *Naegleria* spp. were isolated from various natu-

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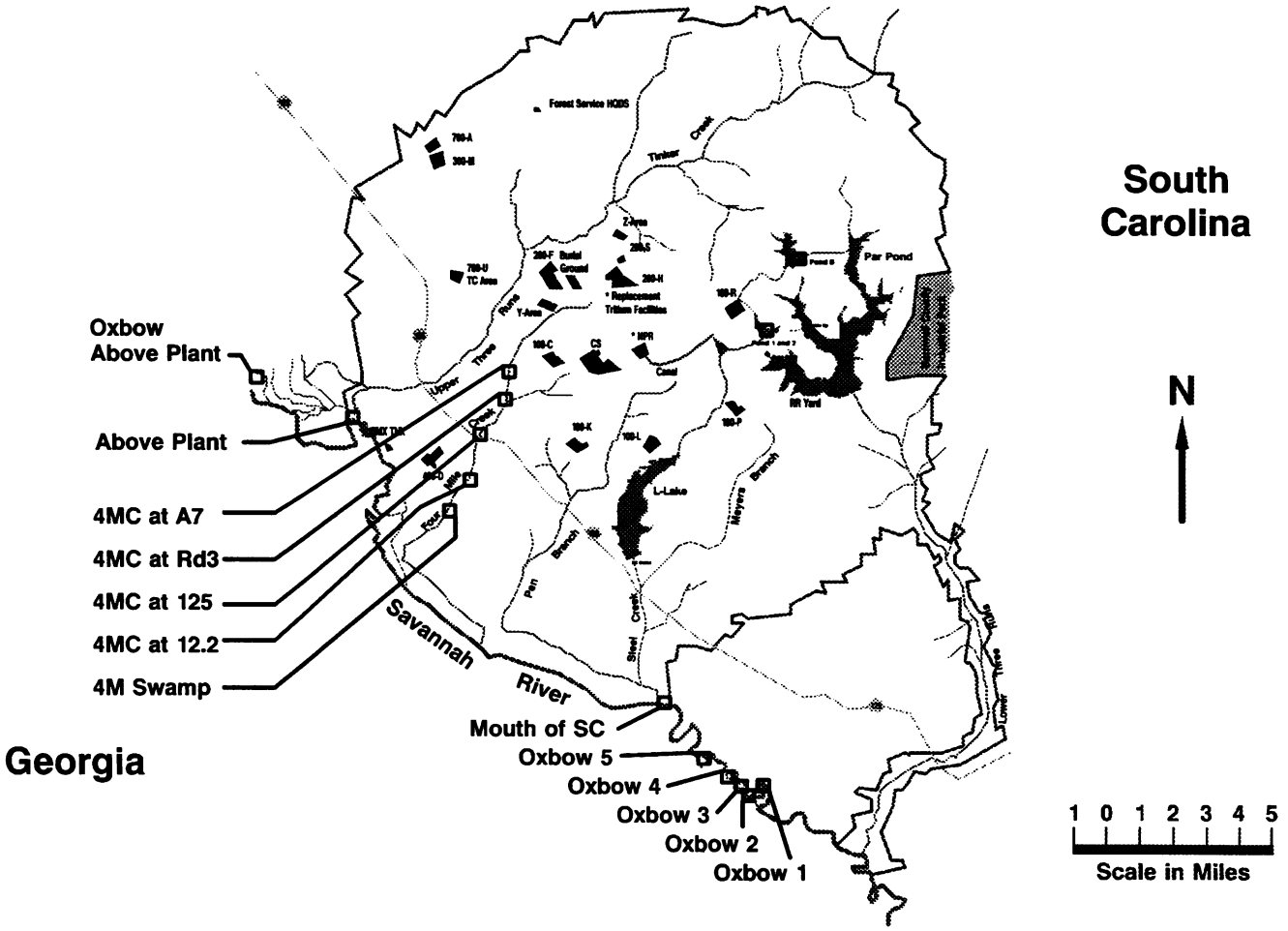

FIG. 1. SRP site map showing sampling locations used for detection of thermophilic amoebae, thermophilic *Naegleria* isolates, and pathogenic *N. fowleri*.

rally heated ponds in South Carolina (10, 13, 14, 21). In studies by Kyle and Noblet (13, 14), *N. fowleri* was shown to be associated with particulate detritus layers in such ponds. An analysis of various thermal springs in New Zealand for *N. fowleri* relative to a variety of habitat parameters was undertaken in 1976 and 1977 (1). Sample sites were generally those associated with previous cases of human meningoencephalitis. Of the total samples tested in 1976, 23% were positive.

An epidemic of 16 deaths between 1962 and 1965 in Czechoslovakia was reported by Cerva (4). After three deaths in 1962 and four in 1963 were traced to an indoor swimming pool, the pool was closed and cleaned. Additional cases appeared after the pool was reopened in 1964, and within days five people became ill and died. After additional analyses, the source of the causative agent, *N. fowleri*, was found sequestered behind repairs previously made on the wall of the swimming pool.

A survey of heated and ambient habitats at the Savannah River plant (SRP) in Aiken, S.C., for thermophilic amoebae in general and thermophilic *Naegleria* spp. in particular was conducted in 1976 (10). Thermophilic *Naegleria* spp. were detected 43% of the time in thermally altered habitats but only 2% of the time in ambient-temperature habitats. The data suggested that thermally altered aquatic systems in the southeastern United States may provide habitats conducive for proliferation of the thermophilic and pathogenic *N. fowleri*.

Individuals living in North Carolina have a higher titer of agglutinating antibodies to thermophilic *N. fowleri* than do those in Pennsylvania, which suggests a greater exposure to these amoebae in the warmer climates (17).

The recent construction of a new reactor cooling lake, L-Lake, at the SRP prompted a new study of the distribution of thermophilic amoebae and pathogenic *N. fowleri* in the newly heated waters. Although previous studies of heated habitats have revealed an obvious qualitative impact of thermal additions on free-living amoebae, to our knowledge little or no quantitative data showing the impact of artificial thermal additions on thermophilic *Naegleria* spp. and pathogenic *N. fowleri* have been obtained. This report documents quantitative results of the impact of thermal additions on the free-living amoeba population of a newly created cooling lake.

MATERIALS AND METHODS

Sampling site. The SRP is a 768-km^2 restricted-access facility operated by the E. I. du Pont de Nemours & Co., Inc., for the U.S. Department of Energy. Within the plant

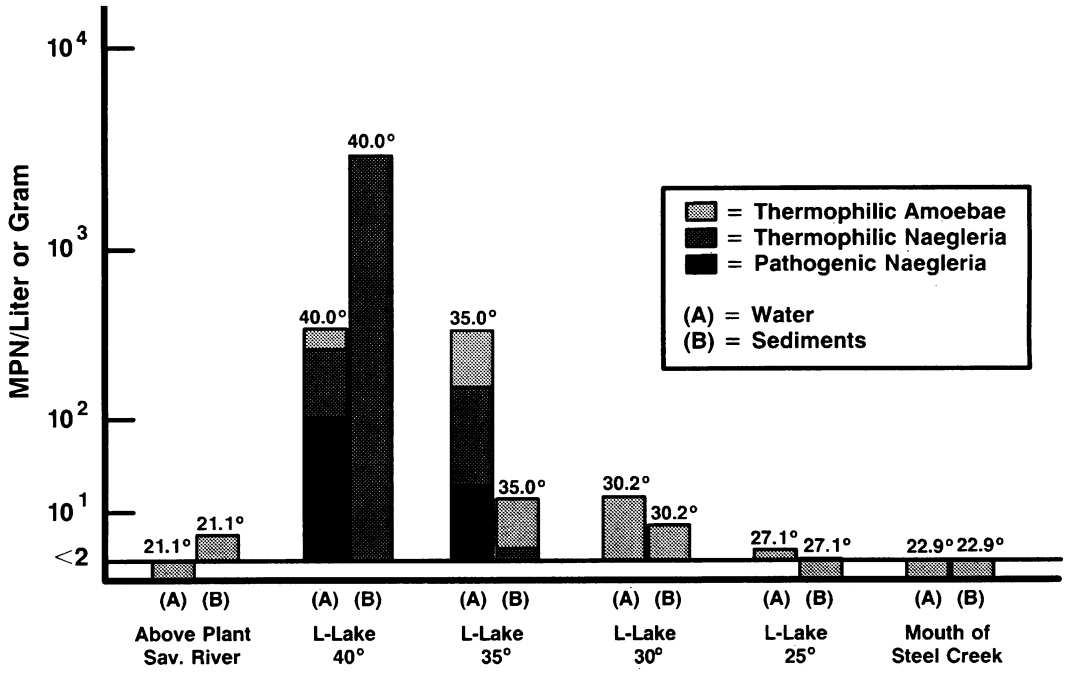

FIG. 2. Amoebic profile in L-Lake in May 1986. MPN, Most probable number.

facility, a 450-hectare cooling lake was constructed in 1985 to receive cooling water discharge from L-reactor, a nuclear production reactor at the SRP. Most of the 450 samples were collected from the water and sediments of L-Lake. Additional samples were collected from L-Lake source water (Savannah River) and discharge waters of L-Lake, Steel Creek (Fig. 1). Thermal additions to L-Lake were initiated in November 1985, and sampling for *Naegleria* spp. began in March 1986. Because of an uneven distribution of the thermal plume, a thermal diversion canal was constructed in the fall of 1986 (Fig. 1).

Amoeba quantification. Analyses for amoebic populations at the SRP site were three tiered. First, the concentration of thermophilic amoebae in general was determined, followed by determination of the subpopulation of thermophilic *Naegleria* spp. Finally, the subpopulation of *N. fowleri*, i.e., pathogenic *Naegleria* sp., was quantified. These thermophilic amoeba populations in environmental samples were detected by plating of small multiple samples.

Five replicates each of 0.01-, 0.1-, 1.0-, 10-, and 100-ml water samples and 0.01-, 0.1-, 1.0-, 10-, and 100-mg sediment samples were tested. The samples were placed on nonnutrient agar plates spread with a lawn of live *Escherichia coli* (NNAE). The 100-ml water samples were filtered through 1.2-μm-pore-size cellulose filters (Millipore Corp., Bedford, Mass.), which were then quartered or halved and inverted onto the NNAE plates. The 10-ml samples were centrifuged at 600 × *g* for 15 min, and the resultant pellets were plated on NNAE plates. The remaining water and sediment samples were placed directly onto the surfaces of the NNAE plates.

The NNAE plates were incubated at 44°C for 7 days or until growth of thermophilic amoebae was observed. These initial outgrowths determined the concentration of thermophilic amoebae. Morphological characteristics typical of the *Naegleria* trophozoite and cyst (i.e., eruptive-like formation of the pseudopodia and smooth cyst wall, respectively) were used to tentatively identify amoebae belonging to the genus *Naegleria*.

All thermophilic *Naegleria* organisms that grew at 44°C with morphology patterns indicative of *Naegleria* spp. were then tested for ability to flagellate. The flagellation tests were performed at 35°C. After being harvested, the trophozoites were suspended in sterile distilled water and examined under an inverted microscope for the presence of flagellates after 1, 2, and 3 h. Results of these analyses determined the concentration of thermophilic *Naegleria* spp.

To determine the concentration of the subpopulation of pathogenic *N. fowleri*, the *Naegleria* organisms that grew at 44°C, demonstrated flagellation, and showed growth indicative of pathogenic *Naegleria* spp. were washed and suspended in sterile distilled water. The suspension (4,000 to 10,000 amoebae) was intranasally instilled in weanling BALB/c mice. When the outgrowth on the original plates was too sparse, the potential pathogens were transplanted to three additional plates and reincubated at 44°C until growth sufficient for pathogenicity tests occurred. Test mice were observed for 2 weeks for signs of encephalitis. Moribund mice were sacrificed, and their brain tissue was plated on NNAE plates. Outgrowth of *Naegleria* spp. from the tissue confirmed the presence of pathogenic *N. fowleri*. Negative controls consisted of weanling mice inoculated with nonpathogenic amoebae, necropsied, and processed as described above.

Each sampling location was measured for temperature, pH, conductivity, and dissolved oxygen levels as determined in situ at the time of sampling, using a HORIBA U-7 meter (Horiba Instruments, Inc., Irvine, Calif.). These data were analyzed for possible correlation with various amoeba subpopulations.

Air sampling. Sites proximal to L-Lake were sampled during periods when the lake was maximally heated and when concentrations of thermophilic amoebae in L-Lake were markedly elevated. Three types of samplers were used. The Anderson sampler impinged airborne microbes directly onto the surface of NNAE plates (12). The air was sampled at a rate of 30 liters/min. Negative controls consisted of agar

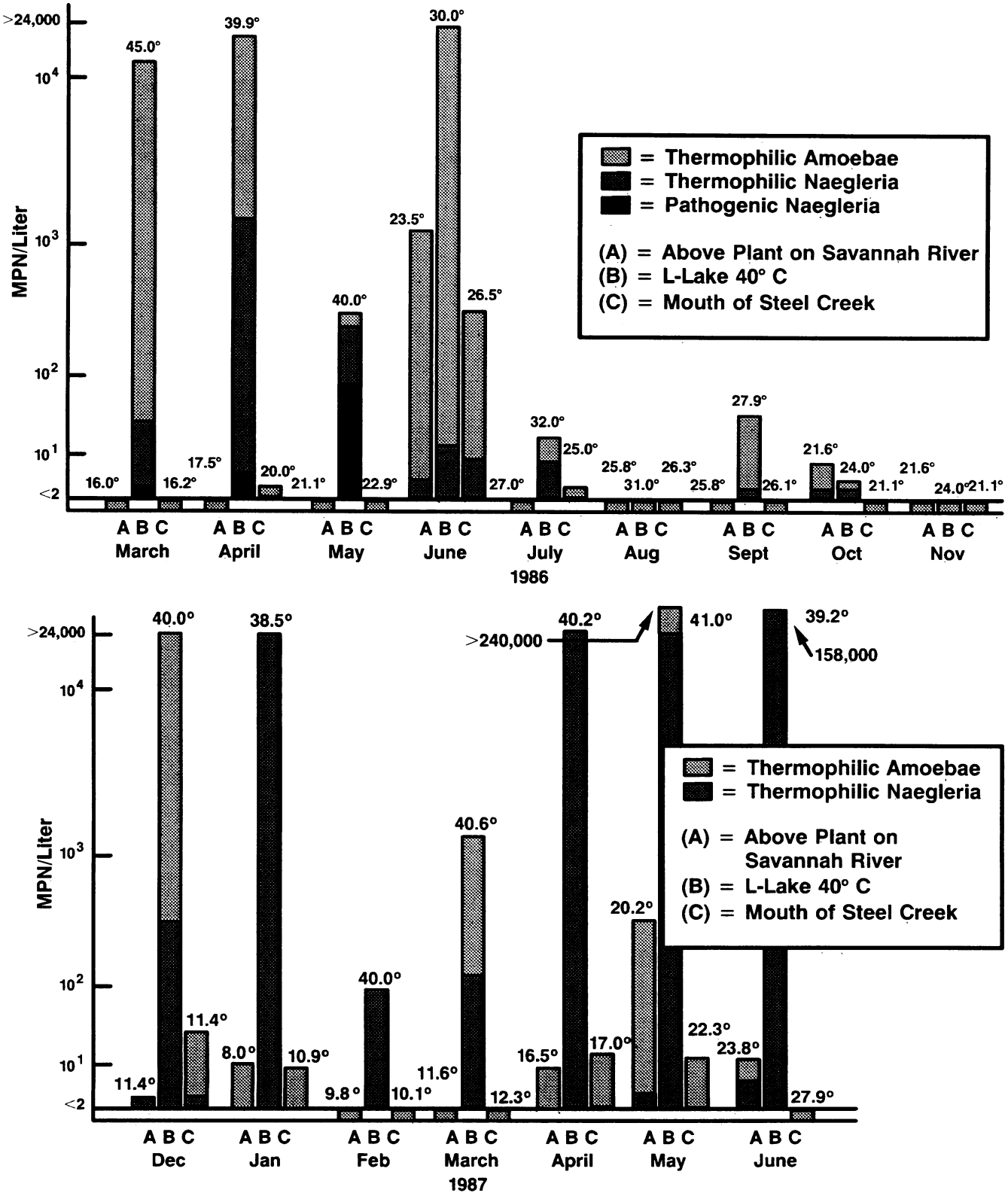

FIG. 3. Seasonal variations of amoebic populations in source water from Savannah River, L-Lake, and Steel Creek. MPN, Most probable number.

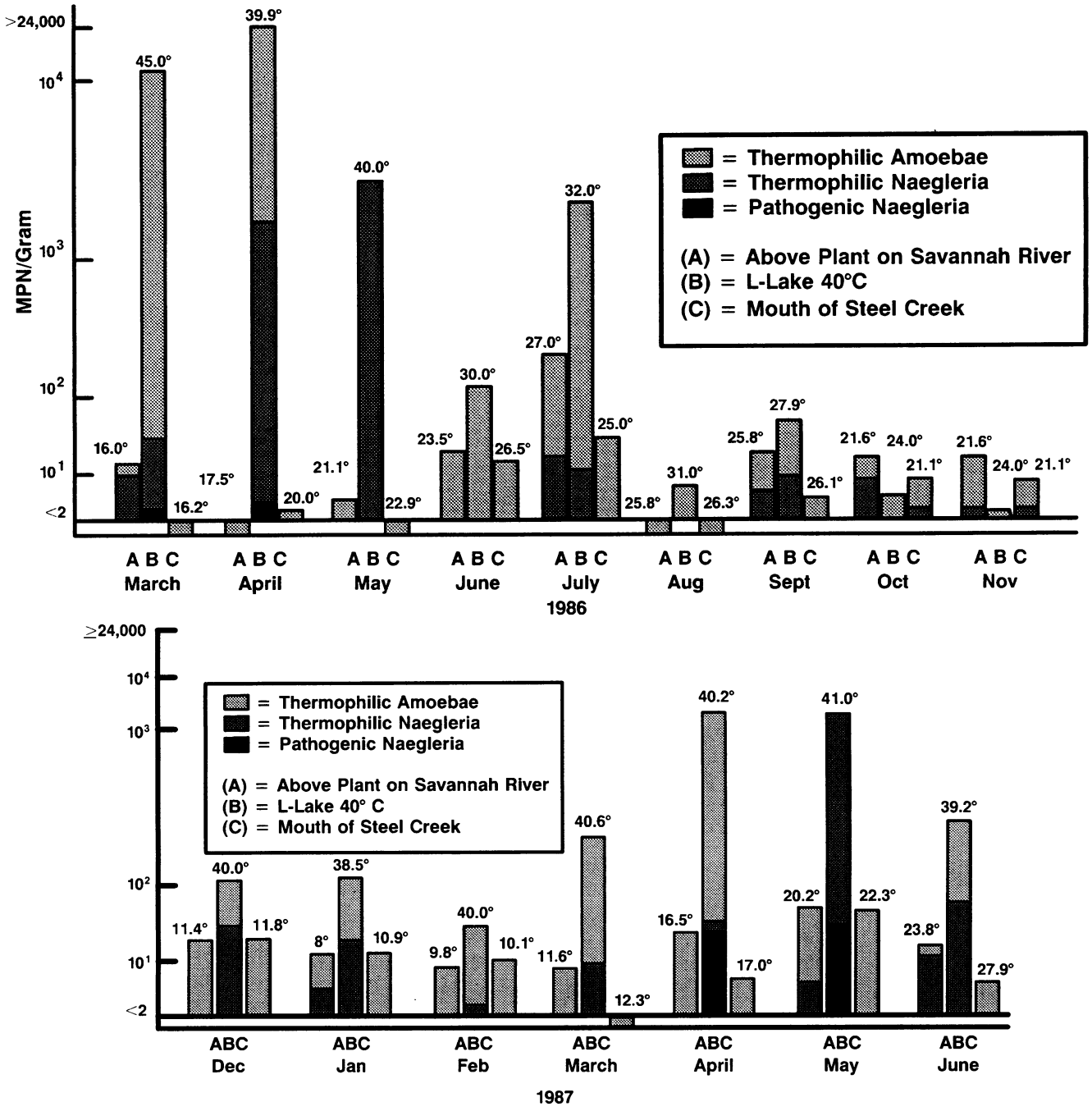

FIG. 4. Seasonal variations of amoebic populations in upriver sediments from Savannah River, L-Lake, and Steel Creek. MPN, Most probable number.

plates inserted into the sampler without the impingement of ambient air.

Greenburg-Smith impinger air samplers were simple two-stage vacuum-drive devices that processed air at approximately 24 liters/min (16). Sampled air was passed through a series of two sterile filtered water reservoirs. The microorganisms that remained in both reservoirs were collected, combined, and analyzed for amoebae as described above. The flow rate of the air samplers was set by using a calibrated rotameter.

A Litton-type high-volume air sampler (16) was also used to collect particulate matter from a large air sample (2.4×10^4 liters) and deposit into 300 ml of sterile water. The collected water was filtered and analyzed for amoebae as described above.

Isoenzyme studies. Pathogenic *N. fowleri* from L-Lake were cultured in a Casitone (Difco Laboratories, Detroit, Mich.)-based medium (CGVS) from infected-mouse brain tissue (26). The axenized *N. fowleri* cultures were pelleted by centrifugation at $500 \times g$ for 10 min, suspended in a distilled water wash, repelleted, and frozen at −40°C. Thawed pellets were suspended in buffered saline (pH 7.2)

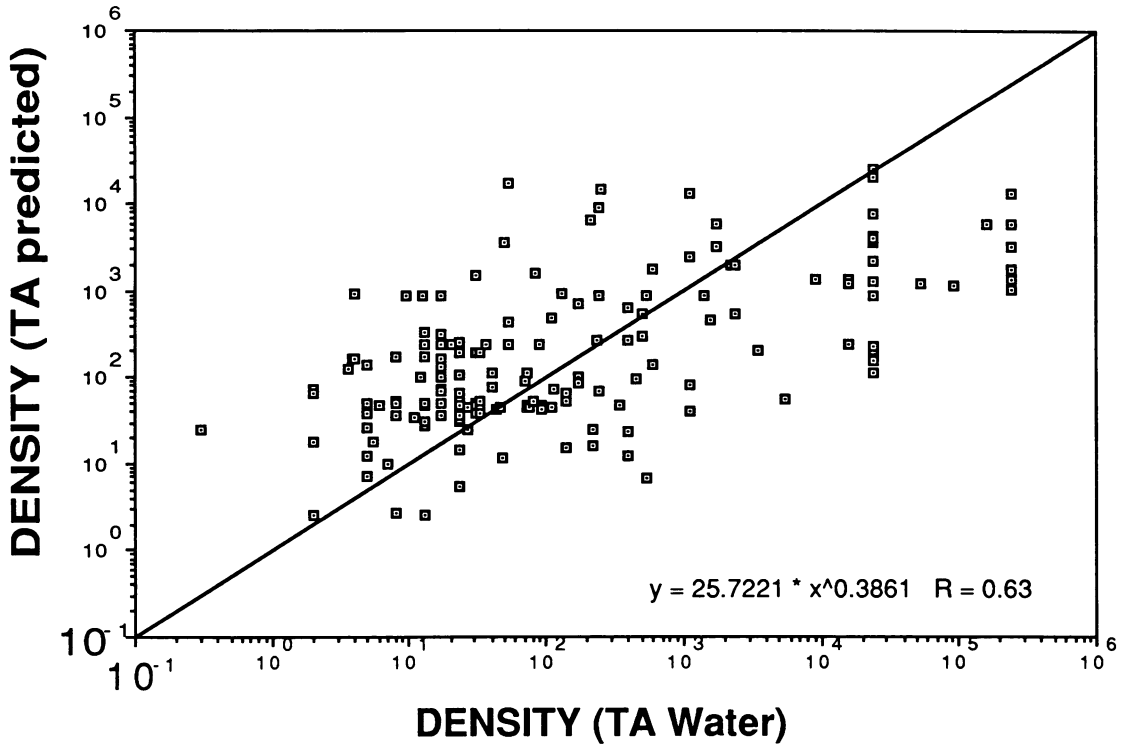

FIG. 5. Densities of thermophilic amoebae (TA) measured in water samples compared with predicted values obtained from independent variables of temperature, pH, and conductivity. The line represents the one-to-one correlation; the actual correlation and the line equation are given.

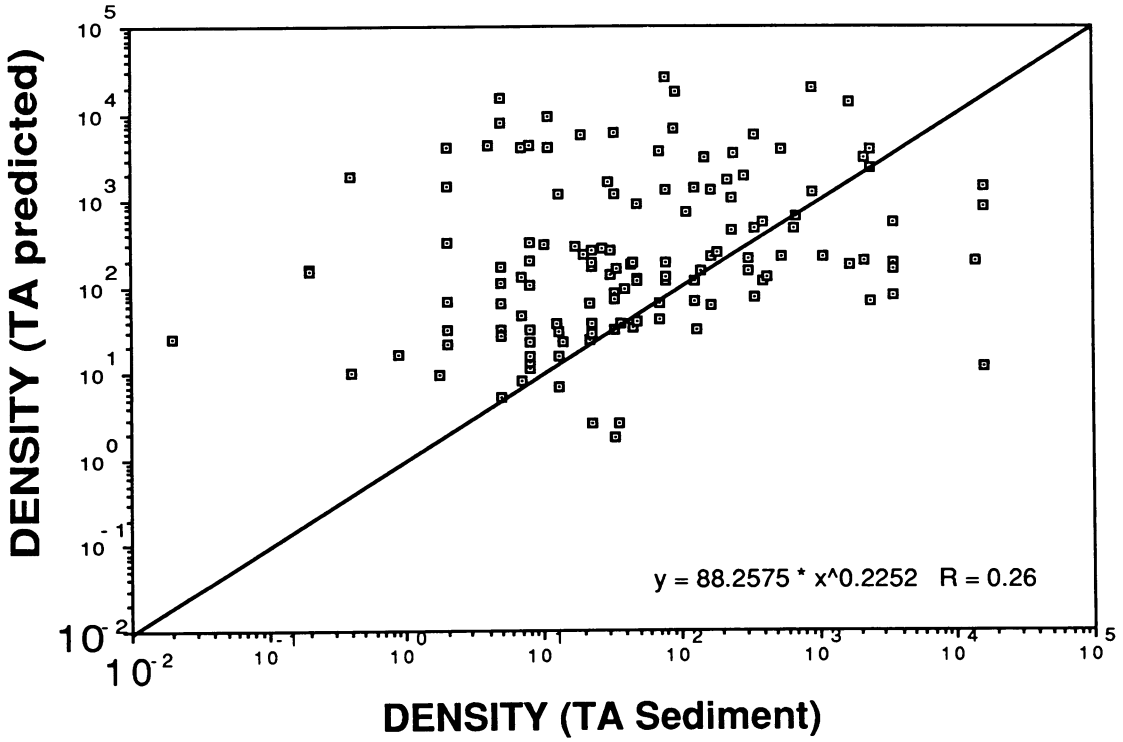

FIG. 6. Densities of thermophilic amoebae (TA) measured in sediment samples compared with predicted values obtained from independent variables of temperature, pH, and conductivity. The line represents the one-to-one correlation; the actual correlation and the line equation are given.

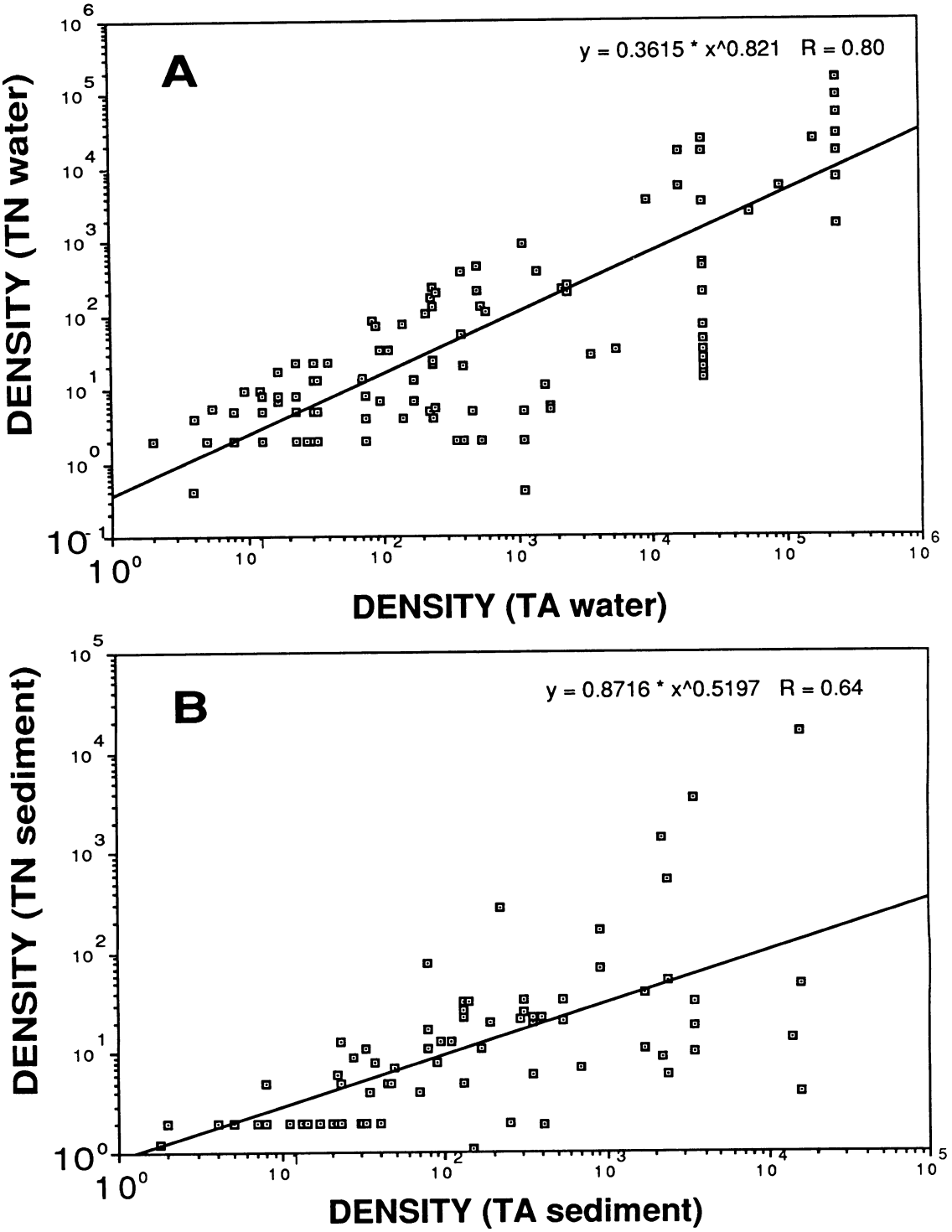

FIG. 7. Relationship between densities of thermophilic amoebae (TA) and thermophilic *Naegleria* isolates (TN). (A) Water samples; (B) sediment samples. The line represents the one-to-one correlation; the actual correlation and the line equation are given.

and electrophoresed in 7.5% acrylamide gel. Isoenzyme profiles of lactate dehydrogenase, L-threonine dehydrogenase, superoxide dismutase, acid phosphatase, malic enzyme, and leucine aminopeptidase were determined as described by Pernin et al. (19). The isoenzyme patterns of the L-Lake pathogen (SRP) were compared with those of known isolates of *N. fowleri* (ATCC 30808) and *N. lovaniensis* (Aq/9/1/45D) obtained from J. DeJonckheere and with those of a thermophilic, nonpathogenic isolate (5049) previously isolated from the SRP site and thought to be *N. lovaniensis* (10).

Data analysis. Prepared programs for the Macintosh II

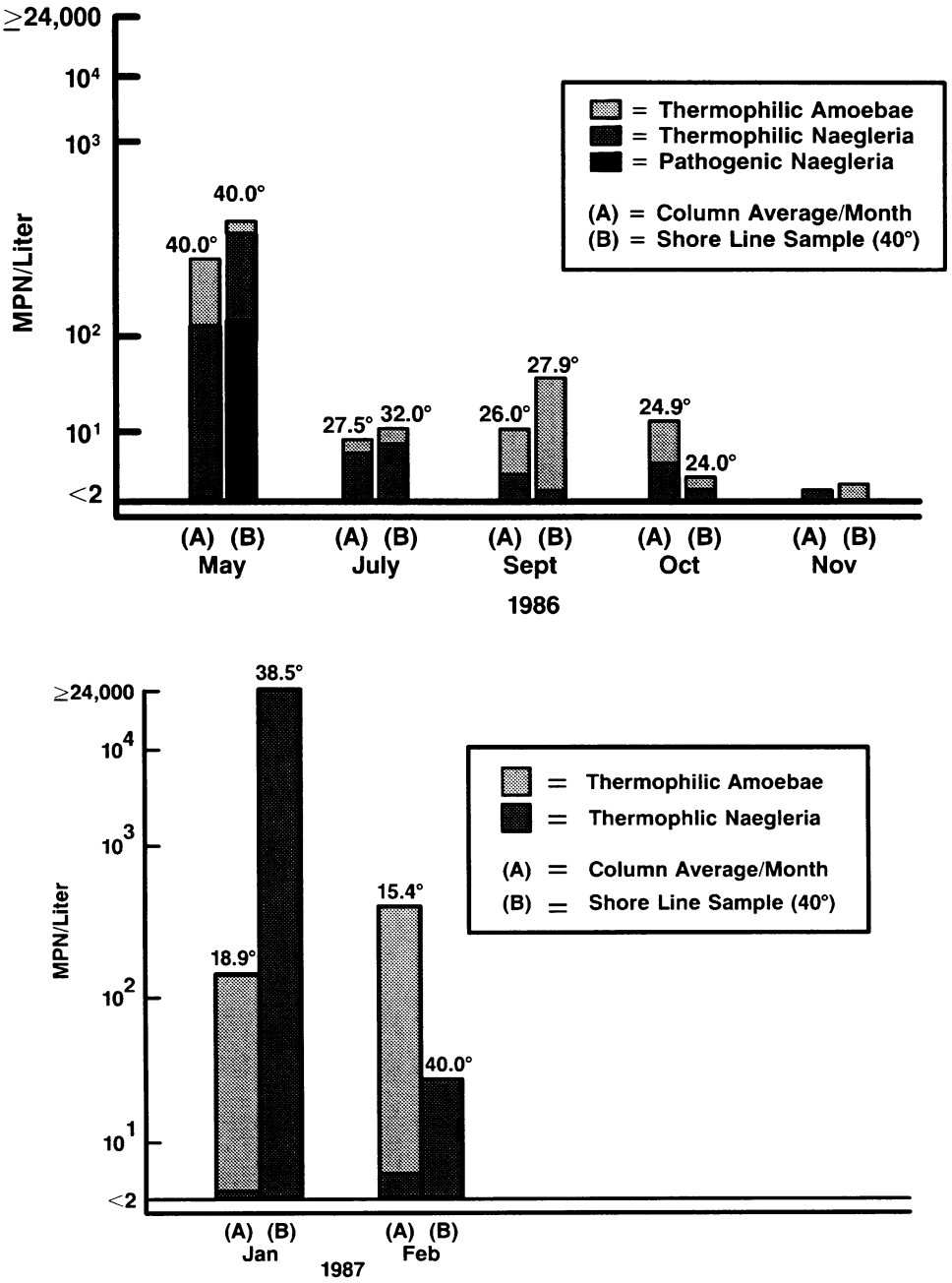

FIG. 8. Comparison of amoebic populations in water column versus shoreline samples. MPN, Most probable number.

computer were used for statistical analysis of data. Best-fit model analyses were done by using the multivariate analysis routines of SYSTAT version 3.1 (SYSTAT, Inc., Evanston, Ill.) Data that were heteroscedastic were transformed with either $\log_{10}(x + 1)$, natural log, or square root according to Zar (27). Probabilities equal to or less than 0.05 were considered significant.

RESULTS

The first thermal additions to L-Lake occurred in November 1985, and samples for thermophilic amoebae were collected 4 months later. Figure 2 shows the results of a typical monthly sample at various points in the thermal gradient on the western shore of the lake as compared with source and discharge samples. These same sampling points were also used when there were no thermal additions from the L-reactor. Figures 3 and 4 show monthly comparisons of source and discharge samples with those at the 40°C sampling site. The thermophilic amoebae and thermophilic *Naegleria* populations increased as much as 5 orders of magnitude in the 40°C samples relative to source water and sediment samples. Pathogenic *N. fowleri* increased as much as 2 orders of magnitude (Fig. 2 to 4) and were detected only in heated lake water or sediment samples; they were not detected in either source or discharge samples.

TABLE 1. Most probable number of thermophilic amoebae in samples from Savannah River oxbows

Site and sample	Most probable no.			
	August 1987		October 1987	
	Per liter of H_2O	Per g of sediment	Per liter of H_2O	Per g of sediment
Upriver[a]				
A	110	5	<2	<2
B	13	40	5	<2
C	13	17	<2	5
D	22	<2	<2	<2
E	7	6	8	2
Downriver[b]				
A	>24,000	49	49	<2
B	94	14	13	49
C	16,000	130	4	170
D	16,000	210	7	23
E	16,000	11	2	79

[a] Upriver from source of water for L-Lake.
[b] Downriver from thermal discharge of SRP from L-Lake. Oxbow configurations and depths at the two sites were similar.

During the 15-month study, the thermophilic amoeba populations in both water and sediment samples persisted as long as thermal additions were present. After the May 1986 sampling, concentrations of thermophilic amoebae, thermophilic *Naegleria* spp. and pathogenic *N. fowleri* dropped within 30 to 60 days after the cessation of thermal input (Fig. 3 and 4). By July and August 1986, the amoebic concentrations in water and sediments were similar to those seen in source and discharge samples. The concentrations of thermophilic amoebae diminished throughout the fall of 1986, during which time no thermal additions in the lake occurred and the thermal diversion canal which redirected the thermal plume to the eastern side of the lake was built. Subsequent thermal additions began in December 1986 and were confined mainly to the thermal diversion canal area. The canal effectively redistributed the thermal plume to the east bank of the lake opposite the canal discharge. Most samples were taken from the shore. In some cases, water column samples were also taken and compared with shoreline samples.

Intermittent thermal additions were present from December 1986 through February 1987 and again produced a marked increase in waterborne thermophilic amoebae and nonpathogenic, thermophilic *Naegleria* spp. During this time, sediments were collected primarily from the riprap bottom of the turbulent thermal diversion canal area at temperatures reflective of prior sampling temperatures along the western shoreline. With more consistent thermal inputs from April through June 1987, concentrations of thermophilic *Naegleria* spp. and pathogenic *N. fowleri* increased in sediment samples taken on the eastern shoreline opposite the discharge point of the thermal diversion canal (Fig. 4).

During the summer of 1987, the concentrations of thermophilic amoebae and thermophilic *Naegleria* spp. were greater in water than in sediments. Waterborne thermophilic amoeba populations were equal to or greater than 2.4×10^5/liter in May, and the thermophilic *Naegleria* population was 1.6×10^5/liter in June samples. Pathogenic *N. fowleri* were isolated from the water in March, April, and May 1986 and from the sediments in March and April 1986 and April and May 1987.

Densities of thermophilic amoebae in water were significantly correlated with temperature ($r = 0.573$, $n = 186$, $P < 0.00001$; range, 8.0 to 72.0°C), conductivity ($r = 0.395$, $n = 186$, $P < 0.001$; range, 40 to 140 μS/cm), dissolved oxygen ($r = -0.481$, $n = 186$, $P < 0.001$; range, 0.6 to 13.0 mg/liter), and densities of thermophilic *Naegleria* spp. ($r = 0.860$, $n = 186$, $P < 0.00001$). Densities of thermophilic amoebae in the sediments were significantly correlated with temperature ($r = 0.279$, $n = 136$, $P < 0.001$), densities of thermophilic *Naegleria* spp. ($r = 0.810$, $n = 136$, $P < 0.00001$), and densities of pathogenic *N. fowleri* ($r = 0.490$, $n = 136$, $P < 0.0001$).

Several models were fit to the data with and without various data transformations, including polynomial, quadratic, exponential, and linear equations. The best-fit model was obtained by using densities of thermophilic amoebae as the dependent variable and temperature, pH, and conductivity as independent variables. These parameters explained 38.4% of the variation in densities of thermophilic amoebae in water ($F = 30.3$, df = 4 and 180, $P < 0.0001$); a fit between actual and predicted values of thermophilic amoebae is presented in Fig. 5. The same model was used to predict densities of thermophilic amoebae in sediments, with moderate success (Fig. 6). The strong relationship between thermophilic amoebae and thermophilic *Naegleria* spp. in both the sediments and water (Fig. 7) suggest that temperature, pH, and conductivity also had a significant effect on the densities of thermophilic *Naegleria* spp. Unfortunately, the small number of samples positive for pathogenic *N. fowleri* did not allow further extrapolation of the model to the pathogen. However, because of the interrelatedness of thermophilic *Naegleria* spp. and pathogenic *N. fowleri*, subsequent data collection should allow further extrapolation.

According to the model and statistical analysis, temperature is the most significant water quality parameter measured in determining the densities of thermophilic amoebae and thermophilic *Naegleria* spp. In addition, conductivity of the water has a strong effect on these two microbial communities in water. The lack of correlation between water conductivity and amoeba densities in the sediment was not surprising, since the sediments are often anaerobic environments, even

TABLE 2. Tests for free-living amoebae in air samples taken near L-Lake

Site	Sampler type[a]	Vol (liters) of air sampled	No. of plates containing amoebae/ 2 plates incubated at:	
			35°C	44°C
Bubble-up	Anderson	900	0	0
	Impinger	720	0	0
	High volume	24,000	0	0
Reactor canal	Anderson	900	0	0
	Impinger	720	0	1[b]
	High volume	24,000	0	0
Steel Creek	Anderson	900	0	0
	Impinger	720	0	0
	High volume	24,000	0	0

[a] Samples were taken as follows: Anderson, 30 liters/min on *E. coli* plates; impinger, 24 liters/min with two rinses before sampling; high volume, 800 liters/min with two rinses before sampling. A 50-ml amount of each high-volume and impinger including rinses, was concentrated to 0.5 ml, placed on two *E. coli* plates, and incubated at 35 and 44°C. The remaining sample (100 to 150 ml) was filtered (1.2-μm-pore-size filter), and each filter was halved and incubated at 35 and 44°C. All rinse samples taken before air samples were negative for amoebae.
[b] Isolate did not flagellate, and morphology was indicative of *Hartmannella* spp. rather than *Naegleria* spp.

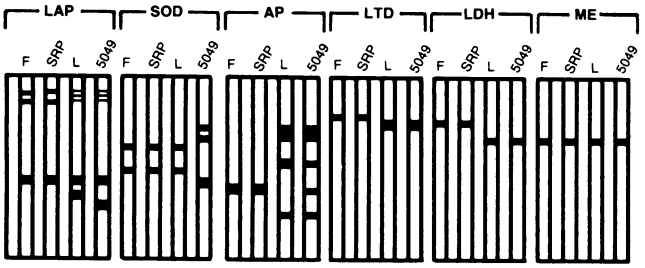

FIG. 9. Comparison of isoenzyme patterns of *N. fowleri* isolated from L-Lake with those of *N. fowleri* and *N. lovaniensis*. LAP, Leucine amino peptidase; SOD, superoxide dismutase; AP, acid phosphatase; LTD, L-threonine dehydrogenase; LDH, lactate dehydrogenase; ME, malic enzyme; F, *N. fowleri* (isolates from the United States and Belgium were identical); L, *N. lovaniensis*; SRP, *N. fowleri* from L-Lake; 5049, nonpathogenic *Naegleria* isolate from SRP.

when the overlying water is oxic. This fact may also explain the weak predictability of the model for sediment amoebae. While perhaps not statistically significant, it was of interest that all but one of the pathogenic *N. fowleri* isolations occurred in spring months (March, April, and May) rather than the hotter period of the year, when the reactor did not operate. Comparisons of water column samples with samples of water taken from shoreline sites with comparable temperatures generally showed little difference in the concentrations of thermophilic amoebae and thermophilic *Naegleria* spp. (Fig. 8). Similarly, differences in amoeba content between samples in the same water column were not observed. However, samples collected in May 1986 contained pathogenic *N. fowleri* from the shoreline but not the water column.

Comparisons of upriver versus downriver oxbow samples in the Savannah River showed increased concentrations of thermophilic amoebae in downriver sites (Table 1). Pathogenic *N. fowleri*, however, were not isolated from either the upriver or the downriver samples.

Air samples taken during periods of thermal input and high concentrations of amoebae showed no evidence of *Naegleria* spp. (Table 2). Only 1 of 36 samples was positive for amoebae.

Pathogenic *N. fowleri* isolated from L-Lake were axenized from the brain tissue of mice, and various isoenzyme patterns were determined. The isoenzyme patterns of the pathogenic *Naegleria* spp. isolated from L-Lake were identical to those of *N. fowleri* isolates from heated waters in Illinois and Belgium but differed from those of *N. lovaniensis* and strain 5049 (Fig. 9).

DISCUSSION

Thermal additions to L-Lake correlated with marked increases of thermophilic amoebae, thermophilic *Naegleria* spp., and pathogenic *N. fowleri* in the lake (Fig. 2 to 4). Evidence of thermal impact was also seen in analysis of downriver versus upriver oxbow samples of the Savannah River, the downriver site having higher concentrations of thermophilic amoebae. Whereas previous studies implicated thermal additions as paramount in fostering the presence of thermophilic *Naegleria* spp., including the pathogen *N. fowleri* (6, 8, 21, 22, 25), this study quantified the impact of artificial thermal additions on pathogenic amoeba populations. Thermophilic amoeba populations increased as much as 5 orders of magnitude as a result of thermal input. In some cases, thermophilic amoebae were found in 0.01-ml samples of lake water. Likewise, thermophilic *Naegleria* spp. and pathogenic *N. fowleri* also increased in some heated sites by 5 and 2 orders of magnitude, respectively. Although we have previously isolated pathogenic *N. fowleri* from 1.0-ml water samples (23), this is the first study in which we have shown the presence of pathogenic *N. fowleri* in 0.1-ml or 0.1-mg samples. In comparison, maximal concentrations of thermophilic *Naegleria* in naturally warmed pond water in South Carolina have been reported at approximately 900/liter (14) versus concentrations exceeding 100,000/liter in L-Lake.

Increased sensitivity for detecting the pathogen is often achieved by plating small sample volumes so that other thermophilic, free-living amoeba will be less likely to overgrow the *N. fowleri* (6). Nevertheless, in this study, the extremely high number of thermophilic amoebae and thermophilic *Naegleria* spp. may have occasionally obscured and interfered with the estimation of pathogenic *N. fowleri*, resulting in an underestimation of the true density of the pathogen. An example of such competition was observed in two samples from the study, in which, in the presence of high concentrations of other thermophilic amoebae, 0.1- or 1.0-ml samples were positive for pathogenic *N. fowleri* but 10 or 100 ml did not yield the pathogen.

Undoubtedly, the high concentrations of other thermophiles masked or outcompeted the *Naegleria* spp. for the available *E. coli*, preventing the isolation of the pathogenic *N. fowleri*. In such cases, the most-probable-number tables showed a concentration of pathogenic *N. fowleri* at only $\leq$200/liter, whereas on the reasonable assumption that the 1.0-,10-, and 100-ml samples were also positive, the concentration would have been $\geq$2,000/liter. Similarly, if the concentration of the pathogenic *N. fowleri* in such samples were calculated by the formula of Cerva (4), which takes into account the problem of interfering amoebae, the concentration would be $\geq$2,000/liter.

It is not known why the majority of pathogenic isolates in 1986 were from water samples whereas in 1987 sediments were the source of pathogenic isolates. However, this fact may reflect the markedly lower concentrations of other thermophilic amoebae in sediments than in water, which would not obscure the presence of the pathogen, or it may reflect an inoculation of the sediments by the water column. Second, the sediments collected in 1987 came from the canal per se or from the eastern shore directly affected by the thermal effluent, whereas the 1986 samples were from a less perturbed site on the western shore of the lake. Finally, the recent establishment of L-Lake may account for the differences observed between the sediments and the water samples in that the lake and its microbial populations were coming into equilibrium. Thermal stratification in the lake was not a factor, since stratification in the cooling lake was poor or nonexistent during the time of sampling. In contrast to the strong correlation between thermal additions and amoeba concentrations, no correlation was seen with pH. Lack of correlation with this parameter has been observed previously (21, 22). Whether an apparent correlation exists between detection of pathogenic *N. fowleri* and the spring season, i.e., March, April, and May, is questionable. These months also coincided with a time when pathogen growth was stimulated by recent impact of the thermal discharge but before ambient temperatures plus the man-made thermal additions had stimulated the growth of amoebic thermophiles to concentrations that interfered with detection of the pathogens.

Although waterborne pathogenic *N. fowleri* are classically the agent of primary amoebic meningoencephalitis, there is one report indicating a possible airborne etiology of the disease (15). Thus, travel and other activities near L-Lake and the finding of pathogenic *N. fowleri* in 0.1-ml water samples prompted sampling for airborne amoebae in the vicinity of L-Lake. No *Naegleria* spp. were found in the air samples, and the one sample that was positive for amoebae yielded what appeared to be *Hartmannella* sp. (Table 2). This is not surprising, since water droplets generated by wave action and large enough to contain *Naegleria* organisms would not be expected to travel any significant distance.

Isoenzyme patterns for specific enzymes were used as an additional measure for identifying the species of the *Naegleria* isolates. The isoenzyme patterns of the axenized microorganisms gave further evidence that the isolate from L-Lake was *N. fowleri*. The isoenzyme patterns of lactate dehydrogenase, L-threonine dehydrogenase, superoxide dismutase, acid phosphatase, malic enzyme, and leucine aminopeptidase were used to distinguish the thermophilic *Naegleria* isolates as described by Pernin et al. (19). The isoenzyme patterns of the L-Lake pathogenic isolate were identical to those of *N. fowleri* isolates from heated sites in Belgium and Illinois but differed from those of *N. lovaniensis* and *Naegleria* 5049 isolates. The marked difference in the profiles of the known *N. lovaniensis* and the presumed *N. lovaniensis* isolate 5049 is interesting. We are currently comparing isoenzyme patterns of isolate 5049 with those of *Naegleria australiensis* for indications of similarity or for indications that 5049 is a previously unidentified *Naegleria* species.

The quantification of thermophilic amoebae, thermophilic *Naegleria* isolates, and pathogenic *N. fowleri* in L-Lake dramatically illustrates the effects of thermal additions on the growth and isolation of these amoebae from a thermally altered lake within months of its creation and thermal loading. Because of the masking of the pathogen by the other microorganisms in the environment, the true response of pathogenic *N. fowleri* to environmental perturbations was not fully appreciated or understood. An investigation using monoclonal antibodies (24) prepared against pathogenic *N. fowleri* in conjunction with flow cytometry (18) is now under way.

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